Effects of Low Temperature on Egg and Larval Stages of the Lesser Appleworm (Lepidoptera: Tortricidae)

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ABSTRACT Lesser appleworm, *Grapholita prunivora* (Walsh), eggs were subjected to cold storage conditions at 2.0°C ± 0.2°C for 0–90 d. The most tolerant embryonic stage was the blackhead stage (96–120-h-old eggs) with an LT_{90} of 25 d. The four instars of lesser appleworm were subjected to cold storage conditions at 2.0°C ± 0.2°C for 0–280 d. The fourth instar was the most tolerant to cold storage, with an LT_{90} of 71.5 d. Exposure to low temperatures such as those commonly used for fruit storage shows promise as an alternative to fumigation for lesser appleworm eggs and larvae on apples and pears after harvest.

KEY WORDS lesser appleworm, *Grapholita prunivora*, Tortricidae, cold, apple

Lesser appleworm, *Grapholita prunivora* (Walsh), is a quarantine pest on pome fruits destined for export markets such as Japan, Mexico, and members of the European Union (OEPP/EPPO 1982, 1988, 1990). To meet quarantine requirements in some markets, inspection and certification that the insect is not present in a shipment of fruit is sufficient. In other markets, a treatment such as a chemical fumigation is required after harvest (Moffitt 1988, Moffitt and Burditt 1989a). Nonchemical treatments, especially those that can be easily incorporated into current postharvest practices and that may be adopted by both conventional and organic processors are most desirable.

Temperature extremes have been studied as potential quarantine treatments for pests in temperate fruits (Moffitt and Albano 1972; Moffitt and Burditt 1989a,b; Neven 1994, 1998; Neven and Mitcham 1995; Neven and Rehfield 1995; Neven et al. 1996; Toba and Moffitt 1991; Yokoyama et al. 1991; Yokoyama and Miller 1987, 1989). Exposure of lesser appleworm eggs to the upper limit of low temperatures commonly used for commercial storage of apples, ≈2°C (Kupferman 1996), is a possible alternative to fumigation as a treatment for lesser appleworm eggs and larvae.

The effects of low temperatures on codling moth, *Cydia pomonella* (L), eggs were reported by Moffitt and Burditt (1989a,b). Based on mortality, the order of tolerance of three embryonic stages of the codling moth eggs for temperatures near 0°C was red ring > white > blackhead (Moffitt and Burditt 1989b). Thirty-six- to 42-d exposure was required for complete mortality on mature 'Red Delicious' or 'Golden Delicious' apples (Moffitt and Burditt 1989b).

An accepted treatment for codling moth, *C. pomonella* for access of U.S. apples to Japan is a 55-d cold treatment at 2.2°C or below followed by fumigation with methyl bromide (32 g/m^3 at 10°C for 2 h) (Moffitt 1988; Hansen et al. 2000, 2002). However, it is not known whether a 55-d cold treatment at 2.2°C would kill the lesser appleworm eggs. Preliminary research (Neven et al. 2004) indicated that the embryonic stages of the lesser appleworm were similar to those of codling moth (Richardson et al. 1982). Therefore, a study was conducted to determine the mortality effect induced by low temperature on the white, red ring, and blackhead stages of embryonic development of the lesser appleworm egg and the period of exposure required to achieve 100% mortality for each egg stage. A second study was conducted to determine the mortality effects of cold treatment on the four instars of lesser appleworm and the period of exposure required to achieve 100% mortality of each stage.

Materials and Methods

Determination of Most Tolerant Egg Stage. Lesser appleworm adults were collected from a laboratory culture reared on thinning apples at 25 ± 1°C, 70% RH, and a photoperiod of 16:8 (L:D) h as described by Mantey et al. (2000). Approximately 500 adults were allowed to oviposit for 24 h at 25 ± 1°C, 70% RH, and a photoperiod of 16:8 (L:D) h on immature organic 'Delicious' apples, the preferred oviposition substrate of lesser appleworm (Mantey et al. 2000), in a cardboard box (61 by 61 by 7.6 cm) (length by width by height) with the bottom covered with plastic sheeting (3 mm) and the top covered with nylon organdy and screen netting held in place with a cardboard top having an opening of 53 by 53 cm. At the end of the 24-h period, the adults were removed, the apples examined, and the position of eggs on the fruit were marked to facilitate evaluation after treatment. Apples

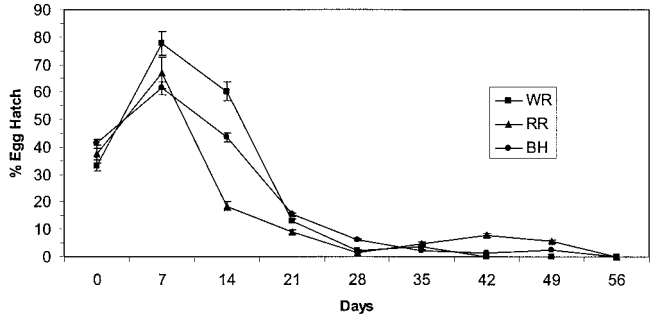

Fig. 1. Percentage of egg hatch (±SEM) of the three embryonic stages of lesser appleworm subjected to cold storage at 2.0 ± 0.2°C. ■, WR, white; ▲, RR, red ring; and ●, BH, blackhead.

with the eggs were held at 25 ± 1°C, 70% RH, and a photoperiod of 16:8 (L:D) h until development to the desired embryonic stage was reached; 0–48 h for white stage, 48–96 h for red ring stage, and 96–120 h for blackhead stage (Neven et al. 2004). The apples were then placed at the experimental temperature of 2 ± 0.2°C for 0–90 d at 7-d intervals. Approximately 200 eggs on the apple surface were used for each replication. Three replications were included for each exposure period.

Replicates were removed from the low-temperature environment at the end of each exposure period and held for at least 9 d at 25 ± 1°C, 50% RH, and a photoperiod of 16:8 (L:D) h for development and hatch of survivors. At the end of the period, the apples were examined under the microscope and each egg was evaluated. The criterion for survival was hatch.

Determination of Most Tolerant Instar. Adult collection and oviposition on fruit were similar to those described previously. However, fruit with eggs were held for longer periods to allow for egg hatch, larval entry into the fruit, and larval development. Infested fruit were held for 8 d for the first instar, 10 d for the second instar, 13 d for third instar, and 16 d for fourth instar (Neven et al. 2004) until treated. Apples with larvae were then placed at the experimental temperature of 2.0 ± 0.2°C for 0–280 d. After apple removal from the treatment, apples were placed at 25 ± 1°C, 50% RH, and a photoperiod of 16:8 (L:D) h for 2–3 d, after which larvae were recovered from the apples by carefully dissecting the fruit with a scalpel and retrieving the larvae with a fine camel's-hair brush or soft forceps. Larval mortality was established by prodding insects with a camel hair brush or soft forceps and observing movement. Insects were considered live when they crawled away from the probing, insects that showed movement of legs, but not mobility, were considered moribund. Moribund larvae were placed on thinning apples and held for 7 d at normal rearing conditions, after which they were evaluated for survivorship. Control fruit were evaluated for proportion of hatched eggs resulting in desired instar. The number of larvae in each treatment group was corrected for control conversion efficiency (hatched egg to larvae).

Statistical Analysis. All data were subjected to an analysis of variance (ANOVA) and Tukey's mean separation (PROC GLM, SAS Institute 2002). Time–mortality responses were estimated by probit analysis of transformed data with PROC PROBIT (SAS Institute 2002). Lethal times to achieve 90 or 99% mortality (LT_{90} and LT_{99} values) were considered to differ significantly if their 95% fiducial limits did not overlap. Slopes were compared with a likelihood ratio test of parallelism ($P = 0.05$) (SAS Institute 2002).

Results and Discussion

Most Tolerant Egg Stage. The effects of cold storage at 2.0 ± 0.2°C on the egg hatch of the three embryonic stages of lesser appleworm are shown in Fig. 1. Cold storage for 56 d results in complete mortality of all three embryonic stages treated. The order of tolerance to low temperature of the three broad embryonic stages of lesser appleworm eggs was blackhead > red ring > white stage. The blackhead stage was significantly more tolerant to the low temperature, with an LT_{99} of 51.9 d, the red ring stage at 44.2 d, followed by the white stage at 39.4 d. The LT_{99} values were significantly different between the all stages (Table 1).

Table 1. Time–mortality response of three embryonic stages of lesser appleworm eggs oviposited of Delicious apples to exposure to low temperatures (2 ± 0.2°C)

Stage	*n*	LT_{90} (95% FL) (d)	LT_{99} (95% FL) (d)	Slope ± SE
White	3,307	23.19 (23.14–23.24)	39.41 (39.28–39.56)	4.534 ± 0.008406
Red ring	5,226	20.43 (20.40–20.46)	44.23 (44.08–44.39)	3.114 ± 0.004941
Blackhead	4,255	24.79 (24.73–24.84)	51.94 (51.75–52.14)	3.252 ± 0.005509

Data transformed (PROC PROBIT, version 8.0, SAS Institute 2002). FL, fiducial limit.

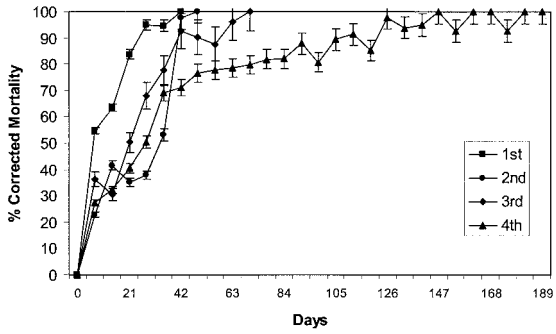

Fig. 2. Percentage of corrected mortality (±SEM) of the four instars of lesser appleworm subjected to cold storage at 2.0 ± 0.2°C. ■, first, first instar; ●, second, second instar; ♦, third, third instar; and ▲, fourth, fourth instar.

These results are in contrast with the results of a similar test with codling moth eggs, in which the red ring stage of the codling moth eggs was determined to be the most tolerant to cold (Moffitt and Burditt 1989b). They also found that the white stage of codling moth eggs was more tolerant than the blackhead stage (Moffitt and Burditt 1989b).

Research on Oriental fruit moth, *Grapholita molesta* (Busck) (Hansen 2002), another internal apple-feeding tortricid, demonstrated that the egg stages were more sensitive to cold treatments than the later instars, but more tolerant than the early instars, requiring 4 wk of cold storage at 1.1°C and 7 wk of cold storage at 3.3°C. It is difficult to compare lesser appleworm egg mortality to this study because developmental stages were not separated and the treatment temperatures were 1.1°C above and below those used in this study. However, the white stage of lesser appleworm did survive >7 wk at 2.2°C, indicating that it is most likely more cold tolerant than the eggs of Oriental fruit moth.

When time–mortality responses of lesser appleworm and codling moth eggs oviposited on 'Delicious' apples are compared, the LT_{90} values for both white (23.2 d) and blackhead (24.8 d) stages of lesser appleworm are greater than those reported for codling moth (17.5 and 10.5 d, respectively) (Moffitt and Burditt 1989b). Dentener et al. (1998) reported an LT_{99} for 1-d-old codling moth eggs, corresponding to the white stage, stored at 2–2.5°C to be 26.2d, whereas we found the LT_{99} value for the same age of lesser appleworm eggs to be 39.4 d. In contrast, the LT_{90} value for the red ring stage of lesser appleworm (20.4 d) was considerably less than that reported for codling moth (26.5 d) (Moffitt and Burditt 1989b). Even though there is a difference in the cold tolerance of the different embryonic egg stages of the two species, based on these results, a cold treatment for a minimum of 55 d at temperatures of 2.0 ± 0.2°C, the currently accepted cold regime used to control codling moth eggs in a two-component treatment accepted for postharvest control of codling moth on apples destined for export markets (Moffitt 1988, Hansen et al. 2000, 2002) should be an effective treatment for postharvest control of lesser appleworm eggs on apples. These data indicate that any cold treatments developed for control of codling moth eggs on host fruits would also be effective in controlling lesser appleworm eggs as well.

Most Tolerant Instar. The effects of cold storage at 2.0 ± 0.2°C on the mortality of the four instars of lesser appleworm are shown in Fig. 2. The order of tolerance to low temperature of the four instars of lesser appleworm was fourth > second ≥ third > first. The fourth instar was significantly more tolerant to the low temperature with a LT_{90} of 71.5 d, followed by the second instar at 37.3 d, the third instar at 36.0 d, and the first instar at 11.2 d. The LT_{90} values were significantly different for all but the second and third instars (Table 2). It is difficult to compare these results with those published for codling moth (Newcomer 1936, Moffitt and Albano 1972, Yokoyama and Miller 1989, Toba and Moffitt 1991) because none of the researchers estimated LT_{90} or LT_{99} levels. Dentener et al. (1998) did report an LT_{99} for diapausing fifth instars of codling moth stored at 2–2.5° to be 81.6 d. However, this estimate was derived from a range of treatments from 0.5 and 10°C. Newcomer (1936) reported that immature larvae of codling moth did not survive 28 d of cold storage (0–1°C), but the actual instars treated were not reported and the total number of larvae treated was extremely low (n = 29). Moffitt and Albano (1972) reported that only diapausing fifth instars of codling moth survived 133 d in controlled atmosphere storage conditions (−0.56 ± 0.28°C, 0.8–1.6% CO_2, and 2.2–3.0% O_2) but that eggs and immature larvae (nondiapausing) did not survive 60 d under these conditions. Again, they did not specify the instars treated, only relative age groups. Toba and Moffitt (1991) did report a Probit 9 ($LT_{99.9968}$) for codling moth larvae exposed to low-temperature controlled atmospheres (0 ± 0.28°C, 1.5–2.0% O_2, <1%CO_2) to be 10.5 wk (73.5 d). Because my estimated LT_{90} for the fourth instar of lesser appleworm was 71.5 d, and our LT_{99} was estimated to be 235.6 d, it seems that lesser appleworm fourth instars may be more cold tolerant than codling moth larvae.

Table 2. Time–mortality response of the instars of lesser appleworm in Delicious apples to exposure to low temperatures (2 ± 0.2°C)

Instar	n	LT_{90} (95% FL) (d)	LT_{99} (95% FL) (d)	Slope ± SE
First	1129	11.24 (11.09–11.38)	46.02 (44.81–47.31)	1.70664 ± 0.01805
Second	1414	37.34 (37.18–37.51)	62.35 (61.90–62.83)	4.6935 ± 0.02795
Third	743	36.04 (37.65–38.38)	56.50 (55.70–57.33)	5.35305 ± 0.04913
Fourth	1078	71.51 (70.16–72.91)	235.66 (226.47–245.66)	2.01740 ± 0.02556

Data transformed (PROC PROBIT, version 8.0, SAS Institue 2002). FL, fiducial limit.

Comparisons of larval cold tolerance to those of Oriental fruit moth are also difficult. Hansen (2002) reported that the early instars of Oriental fruit moth did not survive >6 wk at 1.1°C or 3 wk at 3.3°C, whereas late instars did not survive >10 wk at 3.3°C and 6 wk at 1.1°C. Again, the stages were not separated, nor were LT_{90} values calculated. However, because the early instars of lesser appleworm survived >34 d at 2.2°C, and later instars survived >71 d, it is apparent that lesser appleworm is more cold tolerant than oriental fruit moth. These comparisons indicate the importance to develop an informational database on the relative tolerances of insects potentially infesting a commodity so that appropriate, all-inclusive nonchemical treatments can be developed against the most cold-tolerant species.

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